Stable Anterior Anchoring of the Oocyte Nucleus Is Required to Establish Dorsoventral Polarity of the Drosophila Egg

Guichet, Antoine ; Peri, Francesca ; Roth, Siegfried

Abstract: In Drosophila, dorsoventral polarity is established by the asymmetric positioning of the oocyte nucleus. In egg chambers mutant for cap 'n' collar, the oocyte nucleus migrates correctly from a posterior to an anterior–dorsal position where it remains during stage 9 of oogenesis. However, at the end of stage 9, the nucleus leaves its anterior position and migrates towards the posterior pole. The mislocalisation of the nucleus is accompanied by changes in the microtubule network and a failure to maintain bicoid and oskar mRNAs at the anterior and posterior poles, respectively. gurken mRNA associates with the oocyte nucleus in cap 'n' collar mutants and initially the local secretion of Gurken protein activates the Drosophila EGF receptor in the overlying dorsal follicle cells. However, despite the presence of spatially correct Grk signalling during stage 9, eggs laid by cap 'n' collar females lack dorsoventral polarity. cap 'n' collar mutants, therefore, allow for the study of the influence of Grk signal duration on DV patterning in the follicular epithelium.

DOI: https://doi.org/10.1006/dbio.2001.0354

Developmental Biology **237,** 93–106 (2001)
doi:10.1006/dbio.2001.0354, available online at http://www.idealibrary.com on IDEAL®

Stable Anterior Anchoring of the Oocyte Nucleus Is Required to Establish Dorsoventral Polarity of the *Drosophila* Egg

Antoine Guichet, Francesca Peri, and Siegfried Roth[1]

Universität zu Köln, Institut für Entwicklungsbiologie, Gyrhofstrasse 17, 50923 Cologne, Germany

In *Drosophila*, dorsoventral polarity is established by the asymmetric positioning of the oocyte nucleus. In egg chambers mutant for *cap 'n' collar*, the oocyte nucleus migrates correctly from a posterior to an anterior–dorsal position where it remains during stage 9 of oogenesis. However, at the end of stage 9, the nucleus leaves its anterior position and migrates towards the posterior pole. The mislocalisation of the nucleus is accompanied by changes in the microtubule network and a failure to maintain *bicoid* and *oskar* mRNAs at the anterior and posterior poles, respectively. *gurken* mRNA associates with the oocyte nucleus in *cap 'n' collar* mutants and initially the local secretion of Gurken protein activates the *Drosophila* EGF receptor in the overlying dorsal follicle cells. However, despite the presence of spatially correct Grk signalling during stage 9, eggs laid by *cap 'n' collar* females lack dorsoventral polarity. *cap 'n' collar* mutants, therefore, allow for the study of the influence of Grk signal duration on DV patterning in the follicular epithelium.

Key Words: **oocyte polarity; axis formation; nuclear migration; microtubule network; RNA localisation; *gurken*; *DER*; *pipe*.**

INTRODUCTION

In *Drosophila*, the origin of both major body axes can be traced back to the establishment of oocyte polarity during midstages of oogenesis (van Eeden and St. Johnston, 1999). Oocyte polarisation is a prerequisite for anterior *bicoid* (*bcd*) and posterior *oskar* (*osk*) mRNA localisation and thus determines the anterior–posterior axis (AP) of the later embryo (St. Johnston and Nüsslein-Volhard, 1992). Oocyte polarisation also leads to the asymmetric movement of the oocyte nucleus which defines the dorsal side of the egg chamber and initiates dorsoventral (DV) patterning of both the eggshell and the embryo.

Oocyte polarity arises from reciprocal signalling between the oocyte and surrounding follicle cells (González-Reyes *et al.,* 1995; González-Reyes and St. Johnston, 1994; Roth *et al.,* 1995). Initially, a signal emanates from the small oocyte and induces the adjacent follicle cells to adopt posterior fates. At the molecular level, this is accomplished by the secretion of the TGFα-like Gurken (Grk) protein from the oocyte (Neuman-Silberberg and Schüpbach, 1993; Peri *et al.,* 1999; Queenan *et al.,* 1999; Bökel and S.R., unpublished results), which activates the Drosophila EGF receptor (DER) in posterior follicle cells. Later, the posterior follicle cells signal back to the oocyte employing a still elusive mechanism (González-Reyes and St. Johnston, 1998). Back signalling triggers the reorganisation of the oocyte cytoskeleton (Theurkauf *et al.,* 1992). Microtubule polarity reverses because the signal destabilises posterior microtubule organising centers (MTOCs) while new MTOCs emerge at the anterior cortex (González-Reyes and St. Johnston, 1994; Lane and Kalderon, 1994). This reorganisation is required for the transport of *bcd* and *osk* mRNAs to opposite poles of the oocyte (González-Reyes *et al.,* 1995; Roth *et al.,* 1995).

The integrity and correct polarisation of the microtubule cytoskeleton are also prerequisites for the movement of the oocyte nucleus from a central position at the posterior pole to an asymmetric position at the anterior cortex (Koch and Spitzer, 1983; Peri and Roth, 2000; Saunders and Cohen, 1999). Two types of experiments show that the asymmetric movement of the oocyte nucleus plays an instructive role in determining DV polarity. First, in binuclear oocytes, each nucleus migrates to a different position along the anterior cortex where it induces dorsal follicle cell fates. Thus, the dorsal side of the egg chamber is not determined before the nucleus has moved, demonstrating that nuclear migration presents the first symmetry-breaking step in DV axis for-

[1] To whom correspondence should be addressed. Fax: 49-221-470-5164. E-mail: siegfried.roth@uni-koeln.de.

0012-1606/01 $35.00

mation (Roth *et al.,* 1999). Second, mutations that prevent nuclear movement lead to rotationally symmetric eggs in which the DV axis is parallel to the AP axis. Thus, nuclear movement establishes the orthogonal orientation of the DV axis with regard to the AP axis (Micklem *et al.,* 1997; Newmark *et al.,* 1997; Peri and Roth, 2000).

grk mRNA associates with the nucleus at the anterior corner and this results in a second round of Grk-DER signalling (Neuman-Silberberg and Schüpbach, 1993). In this instance, Grk induces dorsal and represses ventral follicle cell fates, establishing both the DV pattern of the eggshell and the DV axis of the future embryo (Schüpbach, 1987).

Studies of the molecular mechanisms of oocyte nucleus migration in *Drosophila* have so far focussed on similarities to other systems. In *Aspergillus nidulans* and in *Saccharomyces cerevisiae*, directed nuclear movement depends on interactions of microtubules with the cortical dynactin complex via the minus end-directed motor dynein (rewieded by Holzbaur and Vallee, 1994; Efimov and Morris, 1998; Karki and Holzbaur, 1999). In addition to cytoskeletal components, proteins have been identified which might have regulatory rather than structural functions. NudF from *A. nidulans* and PAC1 from *S. cerevisiae* belong to this group. NudF and PAC1 are homologs of Lissencephaly-1 (Lis-1) which has recently been shown to be a functional component of the dynein/dynactin complex in mammalian cells (Xiang *et al.,* 1995; Geiser *et al.,* 1997; Faulkner *et al.,* 2000; Smith *et al.,* 2000).

Evidence that the same machinery is required for oocyte nucleus movement in *Drosophila* comes from studies of a *Drosophila* Lis-1 homolog (*DLis-1*). Hypomorphic alleles of *DLis-1* lead to inefficient migration of the oocyte nucleus and *DLis-1* interacts genetically with both *dynein heavy chain 64C (Dhc64C)* and *Glued*, the *Drosophila* homolog of Dynactin (Lei and Warrior, 2000; Swan *et al.,* 1999). As expected, defects in nuclear migration caused by mutations in *DLis-1* lead to abnormal DV patterning of the eggshell and the embryo (Lei and Warrior, 2000).

Here, we show that, in addition to migration, anchoring of the nucleus at the anterior cortex of the oocyte is an important aspect of DV axis formation. Germline clones of mutations in the gene *cap 'n' collar* (*cnc*) lead to egg chambers in which nuclear migration occurs normally; however, the nucleus does not remain at the anterior cortex. Rather, it slides back towards the posterior pole. Concomitantly, changes in the microtubule network occur and *bcd* and *osk* mRNAs become localised ectopically. The late displacement of the nucleus provides us with the opportunity to investigate how the duration of anterior Grk signalling influences DV patterning in the follicular epithelium.

MATERIALS AND METHODS

Fly Stocks

Oregon R. *FRT82B* cnc^{03921} and *FRT82B* cnc^{5561}. Both *cnc* alleles carry a P-element inserted in the exon common to all three isoforms (McGinnis *et al.,* 1998; Mohler *et al.,* 1995). *FRT82B* ovo^{D1}. *Tub-TauGFP*, *FRT82B* cnc^{03921}. *FRT82B ubi-nls GFP* (gift from Stefan Luschnig). Germline clones were generated by using the FLP/FRT technique (Chou *et al.,* 1993).

Immunohistochemistry and in Situ Hybridisation

Broad-Complex (Deng and Bownes, 1997), *rho* (Bier *et al.,* 1990), *kek* (Ghiglione *et al.,* 1999), *pip* (Sen *et al.,* 1998), *mirr* (McNeill *et al.,* 1997), *grk* (Neuman-Silberberg and Schüpbach, 1993), and *bcd* (Berleth *et al.,* 1988) transcripts were detected by *in situ* hybridisation with digoxigenin-labeled antisense RNA probes as described by Tautz and Pfeifle (1989). *osk* (Ephrussi *et al.,* 1991) transcripts were detected by *in situ* hybridisation with biotin-labeled antisense RNA probes. The hybridisation procedure was performed according to Tomancak *et al.* (1998).

Cnc protein was detected by using polyclonal antibodies against a domain common to the three isoforms (McGinnis *et al.,* 1998). The polyclonal anti-Grk antibodies are described by Peri *et al.* (1999). Antibodies against activated Mad (Tanimoto *et al.,* 2000) were a gift from P. ten Dijke. Antibody stainings were performed as described by Roth *et al.* (1989).

RESULTS

Cap 'n' Collar Function during Oogenesis

In order to identify new genes involved in the polarisation of the egg chamber, we analysed a collection of lethal P-element insertions on the second and third chromosomes (Perrimon *et al.,* 1996). We used the "FLP-DFS" technique (Chou *et al.,* 1993) to induce germline clones and screened the mutant egg chambers for defects in the localisation of *osk* and *grk* mRNAs to detect changes in both AP and DV polarity. We identified one mutation in which the nucleus is randomly positioned within the oocyte, and *grk* transcripts remain associated with the misplaced nucleus (compare Figs. 1A and 1B). This mutation l(3)03921 is caused by insertion of a P element in the gene *cap 'n' collar* (*cnc*; Mohler *et al.,* 1995; Perrimon *et al.,* 1996). Homozygous mutant embryos exhibit a deletion of mandibular and labral derivatives of the head skeleton (Figs. 1C and 1D). This phenotype corresponds to the zygotic cuticular defect of embryos mutant for the gene *cnc* (Mohler *et al.,* 1995). The *cnc* locus codes for three transcripts produced by alternative splicing which give rise to three isoforms of a bZIP transcription factor (McGinnis *et al.,* 1998). They share a common carboxy-terminal domain containing the bZIP motif. Using polyclonal antibodies which specifically recognise a common domain of the three isoforms (McGinnis *et al.,* 1998), we investigated the expression pattern of Cnc during oogenesis. Cnc protein was found in both germline and somatic nuclei, with higher amounts being detectable in the germline (Fig. 1E). Interestingly, Cnc is present at all stages during oogenesis in the oocyte nucleus (see high levels of Cnc protein accumulating in the dorsal-anteriorly localised oocyte nucleus of a stage-9 egg chamber in Fig. 1E).

The effects of *cnc* mutants on oogenesis were analysed after inducing FLP-mediated recombination in *FRT82B*

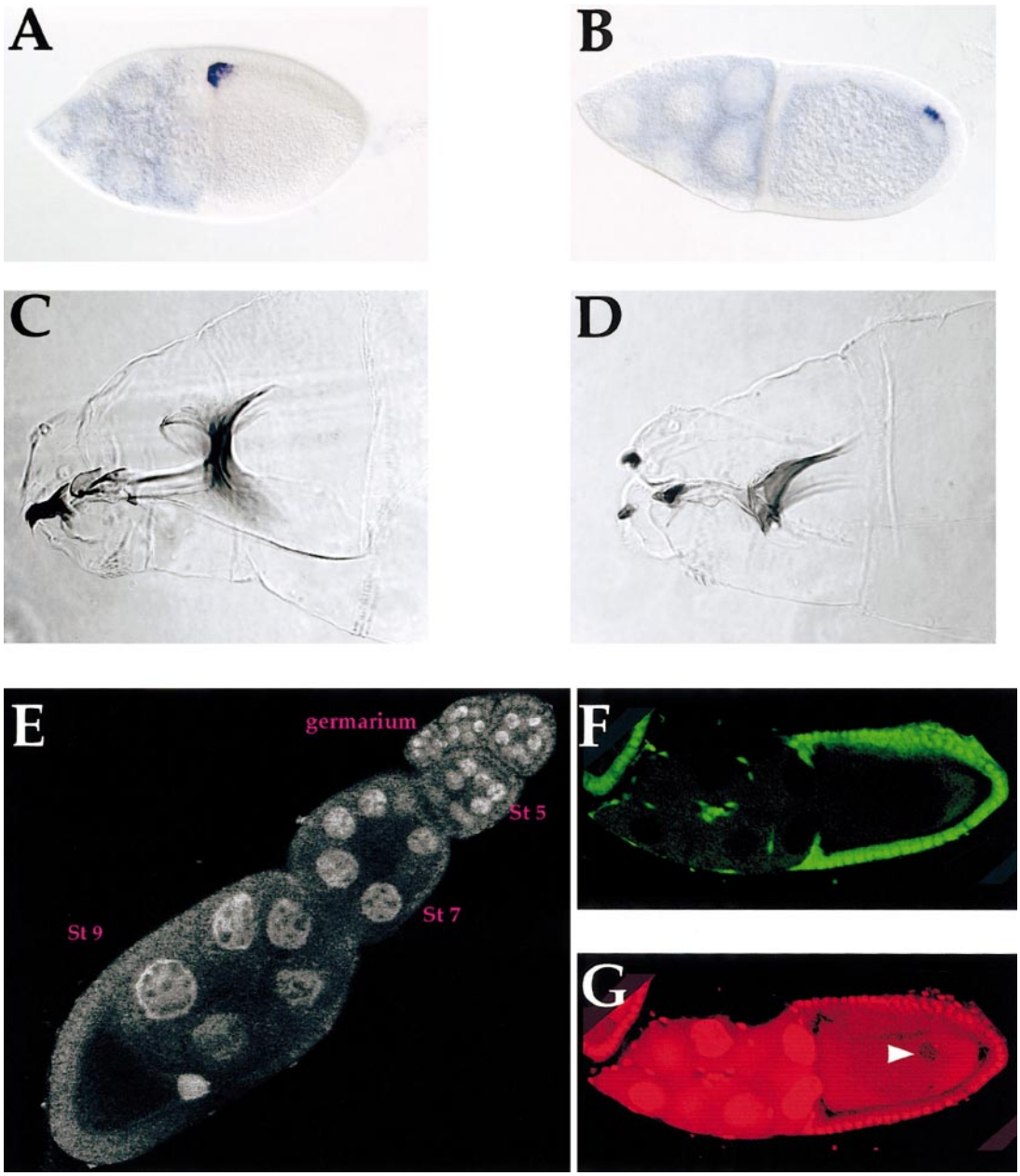

FIG. 1. *cap 'n'collar* mutations affect the localisation of the oocyte nucleus. (A, B) *grk* mRNA distribution in stage-10 egg chambers. (A) Wild-type egg chamber. (B) Mosaic egg chamber with cnc^{03921} mutant germline. (C, D) Phase contrast micrographs showing details of the head skeleton. (C) Wild type. (D) cnc^{03921}/cnc^{03921} mutant larva with deletions of mandibular and labral head structures. Identical phenotypes are observed in larvae mutant for cnc^{05561}/cnc^{05561} and cnc^{05561}/cnc^{03921} (data not shown). (E) Confocal image showing the distribution of Cnc protein during oogenesis. Note the accumulation of Cnc protein in the oocyte nucleus of the stage-9 egg chamber. (F, G) Egg chamber with germline clone after FLP-induced mitotic recombination in *FRT82B ubi-nlsGFP*^{1}*/FRT82B* cnc^{03921} larvae. (F) Only the somatic nuclei show nuclear GFP. The absence of GFP in the nurse cell nuclei and oocyte nucleus indicates that the germline is homozygously mutant for cnc^{03921}. (G) Propidium iodide staining shows all nuclei including the mislocalised oocyte nucleus (arrowhead).

ovo^{D1}*/FRT82B cnc* larvae. Therefore, the term "*cnc* mutant egg chambers" refers to mosaic egg chambers with mostly heterozygous follicle cells and germline cells homozygous for loss-of-function alleles of *cnc*. To make sure that the phenotypes we observed were neither caused by occasional follicle cell clones nor by a dominant interaction between

TABLE 1
Ventralisation Defect of cnc^{5561} and cnc^{03921} Eggs

	Egg shell phenotype (%)					
	Complete ventralisation and wild-type aeropyle	Complete ventralisation and defective aeropyle	Intermediate ventralisation with residual appendage material	Weak ventralisation with one appendage	Wild type	*n*
cnc^{5561}	74	7	3	7	9	117
cnc^{03921}	64	14	8	6	8	120

ovo^{D} and *cnc*, we also induced clones in *FRT82B ubi-nlsGFP*^{1}*/FRT82B cnc* larvae. In this genetic background, the oocyte nucleus was mislocalised only in egg chambers lacking *cnc* in the germline (Figs. 1F and 1G). To assess the influence of genetic background on phenotypic variability, we made clones with two *cnc* alleles (cnc^{03921} and cnc^{05561}) sharing identical amorphic cuticular defects in the embryo (Mohler *et al.,* 1995). We observed the same spectrum of egg chamber defects for both alleles (Table 1, see below for explanation).

The Role of Cap 'n' Collar in Oocyte Nucleus Positioning

In *cnc* mutant oocytes at stage 10, the nucleus is frequently mislocalised, suggesting a role for *cnc* in nuclear migration at stage 8. However, the nucleus is correctly located at the anterior cortex in stage-8/9 *cnc* egg chambers, showing that nuclear migration is initially correct. (Fig. 2A and Fig. 3B). By the end of stage 9, the nucleus is mislocalised in 75% of the *cnc* oocytes ($n = 120$) (Figs. 2 and 3C–3E). Optical cross sections of the oocyte stained with propidium iodide and phalloidin reveal that the mislocated nucleus is tightly associated with the cortical actin network, indicating that it remains attached to the plasma membrane of the oocyte (Figs. 2D and 2E). The observation that *grk* mRNA is always tightly associated with the mislocalised nucleus (Fig. 1B) allowed us to use *grk* mRNA as a probe to follow the nucleus in *cnc* mutant egg chambers. In egg chambers older than stage 9, the nucleus could be found at all positions along the AP axis of the oocyte (Figs. 3C–3E; for numbers, see figure legend). We therefore conclude that *cnc* affects anchoring of the nucleus to the anterior cortex of the oocyte.

Cap 'n' Collar Affects bcd and osk mRNA Localisation

The distribution of *bcd* and *osk* transcripts is often altered in mutants that affect the position of the oocyte nucleus (González-Reyes *et al.,* 1995; Roth *et al.,* 1995; Swan and Suter, 1996; Swan *et al.,* 1999). *bcd* transcripts were correctly localised along the anterior margin of *cnc* oocytes until the end of stage 9 (Figs. 4A and 4B). However, by stage 10, an additional cortical ring of *bcd* mRNA is found at variable AP positions in 85% ($n = 51$) of *cnc* mutant oocytes (Fig. 4D). This distribution of *bcd* is different from that observed in *grk* mutant oocytes where *bcd* mRNA is located to the anterior and posterior extremities of the oocyte (González-Reyes *et al.,* 1995; Roth *et al.,* 1995). It is interesting to note that, in *cnc* oocytes, the ectopic site of *bcd* localisation frequently (60% of the 51 egg chambers scored) coincides with the misplaced oocyte nucleus (Fig. 4D).

The localisation of *osk* mRNA is also affected in *cnc* mutant oocytes. In wild-type oocytes, *osk* mRNA localisation is very dynamic (Ephrussi *et al.,* 1991; Kim-Ha *et al.,* 1991). Before nuclear migration (stage 7), *osk* mRNA is observed around the oocyte nucleus. After migration (between stages 7 and 8), *osk* is present transiently along the anterior margin, before accumulating in the middle and then to the posterior pole of the stage-9 oocyte (A.G., unpublished results; Figs. 4E and 4G). In *cnc* oocytes, *osk* mRNA is frequently found along the anterior margin as late as stage 9, something never observed in wild type (compare Figs. 4E and 4F). Later, *osk* mRNA does not reach the posterior pole in 70% of *cnc* mutant oocytes ($n = 41$), and becomes localised as a cortical ring around the misplaced oocyte nucleus (Figs. 4H and 4J). This distribution is distinct form *osk* mRNA localisation in *grk* mutant egg chambers where the mRNA is concentrated in the center of the oocyte and often forms a spherical aggregate (compare Figs. 4I and 4J). These results suggest that the aberrantly placed nucleus in *cnc* mutant egg chambers defines new sites of cortical *bcd* and *osk* mRNA accumulation (Figs. 4D, 4H, and 4J).

Cap 'n' Collar Affects Microtubule Distribution

Movement of the oocyte nucleus and mRNA localisation rely on the polarisation of the microtubule network in the oocyte and these processes can be disrupted by chemical inhibitors of microtubule polymerisation (Koch and Spitzer, 1983; Saunders and Cohen, 1999; Peri and Roth, 2000) and mutations impairing microtubule polarity in the oocyte (Lane and Kalderon, 1994; González-Reyes *et al.,* 1995; Roth *et al.,* 1995). Therefore, we visualised the microtubule cytoskeleton in *cnc* mutant oocytes by following the distribution of a Tau-GFP fusion protein (Micklem *et al.,* 1997) known to associate with microtubules. In wild-type egg

chambers, three phases of microtubule organisation can be distinguished during midoogenesis (Theurkauf *et al.,* 1992; Theurkauf, 1994). Before stage 7, microtubules emanate from an organising center (MTOC) located at the posterior of the oocyte. During stages 7 and 8, signalling from posterior follicle cells to the oocyte results in destabilisation of this MTOC which leads to a depletion of microtubules at the posterior pole of the oocyte (Fig. 5A). At the same time, microtubules start to nucleate along the anterior margin of the oocyte so that the overall polarity of the microtubule network is inverted (Fig. 5A). This microtubule organisation is maintained until stage 10A (Fig. 5C; Theurkauf *et al.,* 1992; Theurkauf, 1994).

At stage 8, *cnc* mutant oocytes show microtubule nucleation along the anterior margin and absence of microtubules at the posterior pole (Fig. 5B), a distribution that is indistinguishable from wild type (Fig. 5A). We therefore conclude that the early reorganisation of the microtubule network is normal in *cnc* oocytes. However, by stage 10A, a large number of cortical microtubules can be seen in the posterior half of the oocyte often in the vicinity of the misplaced nucleus (Fig. 5D) whereas the cortical microtubule density is reduced at the anterior pole when compared to wild type (Figs. 5C and 5D). This distribution of microtubules in *cnc* mutant oocyte correlates with that of *bcd* mRNA, most of which is found in a posterior ring while low amounts remain anteriorly. Taken together, these results indicate that the polarity of the microtubule network is established normally during stage 7/8 in *cnc* mutant oocytes and is subsequently lost by stage 10A when the oocyte nucleus leaves its anterior position.

Cap 'n' Collar Mutant Eggs Are Ventralised

Females mutant for *cnc* lay eggs with completely ventralised chorions (about 80%) (Fig. 6 and Table 1), a phenotype suggesting defects in Grk signalling. Some eggs exhibit ectopic dorsal appendage material at more posterior positions (Fig. 6D and Table 1). Some completely ventralised eggs also have an aeropyle defect (Figs. 6E and 6F). As *grk* mRNA is correctly localised until stage 9 in *cnc* mutant ovaries, this strong ventralisation of *cnc* eggshells was surprising and suggested that the translation of *grk* mRNA is impaired. However, antibody staining showed that the amount and localisation of Grk protein in *cnc* stage-9 egg chambers is indistinguishable from wild type (compare Figs. 6G and 6H). When the nucleus is mislocalised in *cnc* stage 10A oocytes, Grk protein continues to be present at the new site of nuclear localisation (Fig. 6J, see wild type in Fig. 6I for comparison). Thus, the ventralisation of *cnc* eggs is due neither to lack of *grk* transcription nor to lack of *grk* translation.

Grk Signalling after Stage 9 Is Required to Establish Dorsoventral Asymmetry

Since Grk protein is present in *cnc* egg chambers, we investigated whether the Grk signal is received by the overlying follicle cells. To this end, we studied whether the follicular epithelium exhibits marker gene expression characteristic of DV patterning. Two types of responses are observed in the follicular epithelium following activation of DER by Grk: an immediate response and one which requires the integration of Grk signalling with other signalling pathways (Wasserman and Freeman, 1998; Peri *et al.,* 1999; Dobens *et al.,* 2000; Peri and Roth, 2000).

The DER pathway-inhibitor *kekkon1* (*kek1*; Ghiglione *et al.,* 1999) is a primary target of Grk signalling. In stage-9 *cnc* egg chambers, *kek1* is activated in follicle cells adjacent to the oocyte nucleus (Fig. 7C). This response is indistinguishable from wild type (Fig. 7A), indicating that *cnc* egg chambers secrete Grk which activates DER in the follicular epithelium. In wild-type stage-10 egg chambers, *kek1* expression becomes more intense at the dorsal–anterior corner (Fig. 7B). In *cnc* mutant egg chambers, the nucleus leaves its dorsal–anterior position at this stage. Only residual *kek1* expression remains at the anterior side, presumably in a position where the nucleus had resided earlier (compare Figs. 7B and 7D). Rarely a new patch of *kek1* expression is found close to the mislocalised nucleus (Fig. 7D).

In contrast to *kek1*, the expression of *rhomboid* (*rho*; Ruohola-Baker *et al.,* 1993) and *Broad-Complex* (*BR-C*; Deng and Bownes, 1997) is dependent on the integration of Grk signalling with Decapentalplegic (Dpp) signalling emanating from anterior follicle cells (Twombly *et al.,* 1996; Dobens *et al.,* 2000; Peri and Roth, 2000). *rho* and *BR-C* are required for patterning and specification of the dorsal appendages, respectively (Deng and Bowns, 1997; Wasserman and Freeman, 1998). In wild-type stage-10 egg chambers, *rho* is expressed in a Grk-independent way in centripetal follicle cells and in a Grk/Dpp-dependent way in dorsal follicle cells where its domain resolves into two closely juxtaposed stripes (Fig. 8A; Ruohola-Baker *et al.,* 1993; Peri *et al.,* 1999). *BR-C* is expressed in two dorsolateral patches of follicle cells from which the dorsal appendages emerge (Fig. 8C). In *cnc* egg chambers, *rho* expression is only observed in centripetal follicle cells (Fig. 8B) and in most egg chambers *BR-C* expression is completely absent (data not shown). Occasionally, ectopic *BR-C* expression was seen close to the misplaced nucleus (Fig. 8D). These expression patterns explain the absence of dorsal appendages in most *cnc* eggs and the small number of eggs exhibiting posteriorly shifted dorsal appendage material. They further imply that Grk signalling to dorsal–anterior follicle cells at stage 9 is not sufficient to initiate the expression of genes which besides Grk require Dpp signalling for their activation.

To identify the follicle cells in which the Dpp signal is actually received, we used an antiserum against the activated form of the Mad (pMad; Persson *et al.,* 1998; Tanimoto *et al.,* 2000). Mad is one of the cytoplasmic signal transducers of the Dpp pathway (Raftery *et al.,* 1995; Wiersdorff *et al.,* 1996). In stage-8 egg chambers, high levels of pMad are restricted to a ring of anterior

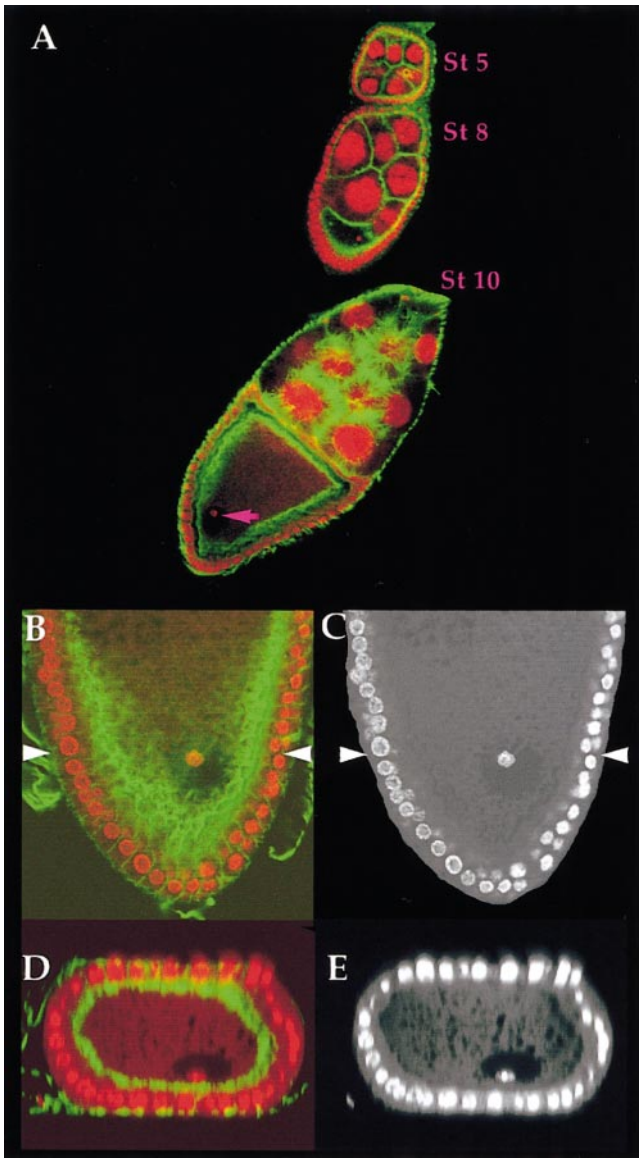

FIG. 2. Oocyte nucleus mislocalisation in *cnc* mutant egg chambers. In 75% of all stage-10 egg chambers ($n = 120$) missing cnc^{03921} activity in the germline, the oocyte nucleus is mislocalised. Confocal images with actin detected by FITC-labelled phalloidin (green) and DNA by propidium iodide (red). (A) Ovariole with three egg chambers. (B, D) Magnified stage-10A egg chamber in which the oocyte nucleus is mislocalised to the posterior pole. (C, E) Only the propidium iodide labelling is shown. (D, E) Optical z section taken at the level of the white arrowheads in (B) and (C). The mislocalised nucleus remains associated with the cortex.

follicle cells which is separated from the nurse cell oocyte border by a zone of follicle cells without detectable pMad (Fig. 9A). During stage 9, the pMAD-positive cells migrate posteriorly (Fig. 9B) and at stage 10 reach the nurse cell oocyte border where the oocyte nucleus resides in wild-type egg chambers (Fig. 9C). At this stage, the oocyte nucleus has left its anterior–dorsal position in most of the *cnc* egg chambers. Consequently, it might not be possible in *cnc* egg chambers to establish a zone in which Grk and Dpp signalling inputs overlap to initiate anterior–dorsal follicle patterning.

It has been suggested that Grk signalling to dorsal follicle cells leads to repression of *pipe* via activation of the homeobox transcription factor Mirror (Jordan *et al.,* 2000). *pipe* codes for an extracellular matrix (ECM) modifying enzyme which is required in a stripe of ventral follicle cells for embryonic DV axis formation (Sen *et al.,* 1998). Since *cnc* eggs do not support embryonic development, a direct observation of embryonic DV patterning is impossible. However, the expression patterns of *mirror* and *pipe* should indicate whether *cnc* egg chambers possess the prerequisites to form a correct embryonic DV axis. Interestingly, although early Grk signalling leads to DER activation as *kek1* expression demonstrates, *mirror* is not activated at the dorsal side (compare Figs. 8E and 8F) and *pipe* is

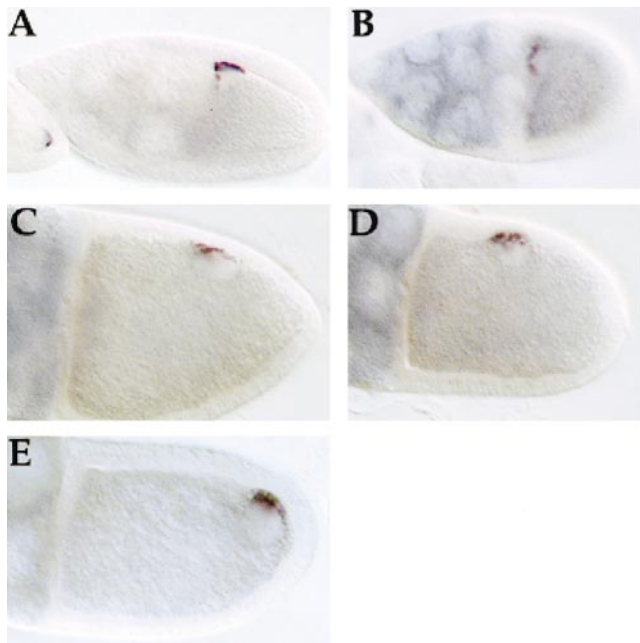

FIG. 3. Randomised misloclisation of the oocyte nucleus in *cnc* mutant egg chambers. (A–E) *grk* mRNA distribution indicates the position of the oocyte nucleus. (A) Wild-type stage-9 egg chamber. (B) cnc^{05561} mutant stage-9 egg chamber. The oocyte nucleus is correctly localised. (C–E) Different positions of the nucleus in cnc^{0556} stage-10 egg chambers. Among 80 stage-10 egg chambers analysed, the nucleus was close to the anterior pole in 19 egg chambers, in a central position posterior to the midline of the oocyte in 22 egg chambers (C), in a central position anterior to the midline of the oocyte in 20 egg chambers (D), and close to the posterior pole in 19 egg chambers (E). This distribution indicates that nuclear postion in *cnc* egg chambers is randomised after stage 9.

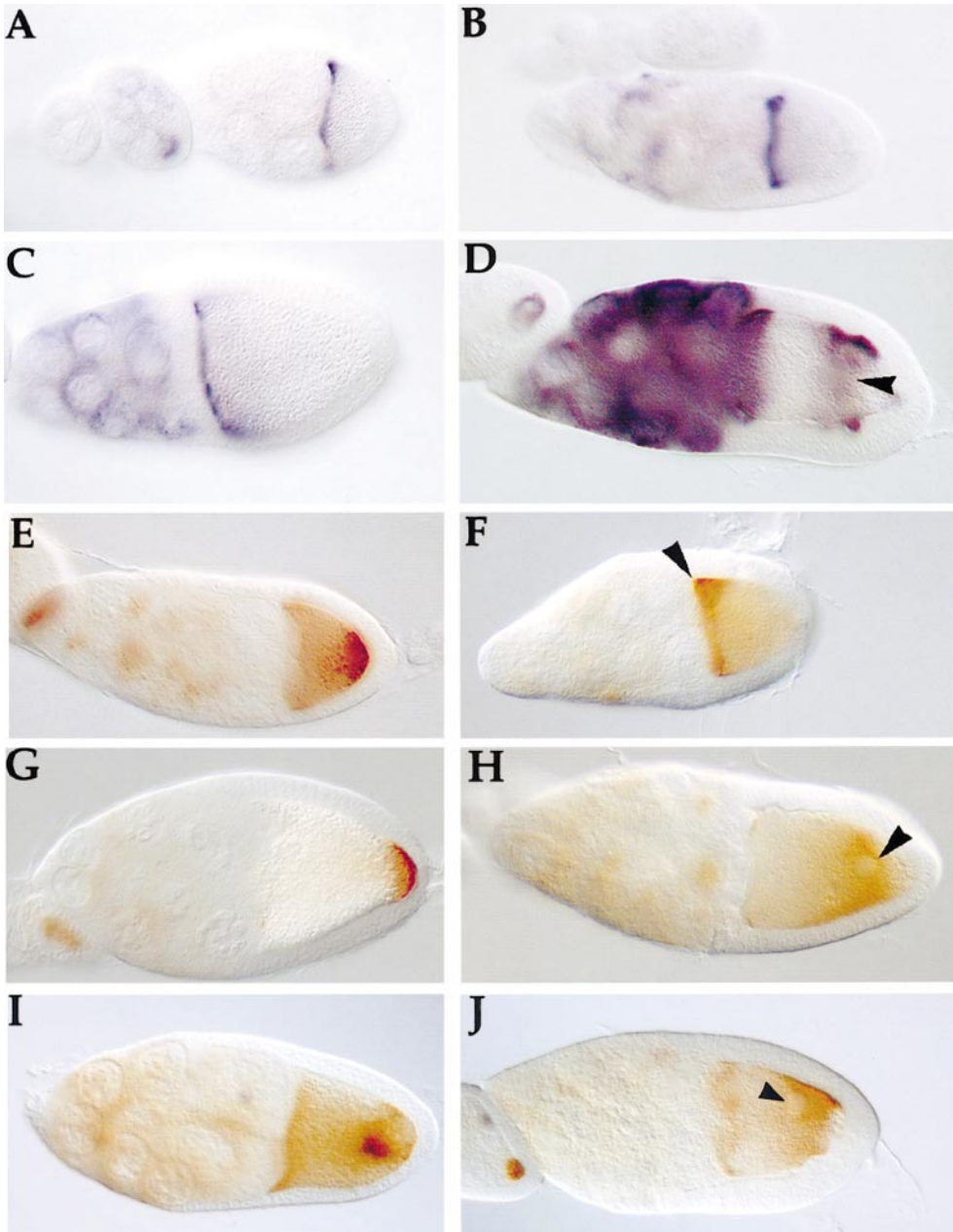

FIG. 4. *cnc* affects *bcd* and *osk* mRNA localisation. (A–D) *bcd* mRNA distribution. (A, C) Wild-type stage-9 (A) and stage-10 (C) egg chambers. (B, D) cnc^{05561} mutant egg chambers. (B) Stage 9. *bcd* mRNA distribution is like in wild type (A). (D) Stage 10. *bcd* mRNA is present as an anterior ring and as a ring in the posterior part of the oocyte close to the mislocalised nucleus (black arrowhead). (E–J) *osk* mRNA distribution. (E, G) Wild-type stage-9 (E) and stage-10 (G) egg chambers. (I) grk^{DC}/grk^{2B6} mutant late stage-9 egg chamber. (F, H, J) cnc^{0556}mutant egg chambers. (F) Stage 9. *osk* mRNA accumulates along the anterior margin of the oocyte. (H, J) Stage 10. *osk* mRNA is mislocalised as a ring in the posterior part of the oocyte at the position of the nucleus (black arrowhead). In *cnc* mutant egg chambers, *osk* mRNA concentrates at the cortex (H, J), in contrast to *grk* mutant egg chambers in which *osk* mRNA accumulates in the center of the oocyte (I).

uniformly expressed in the follicular epithelium of stage-10A egg chambers (Figs. 8H and 8J). Thus, Grk signalling must extend at least until stage 10A in order to establish the ventral *pipe* domain. In summary, Grk signalling being limited to stage 9 in *cnc* egg chambers is neither sufficient to provide the patterning information for DV chorion struc-

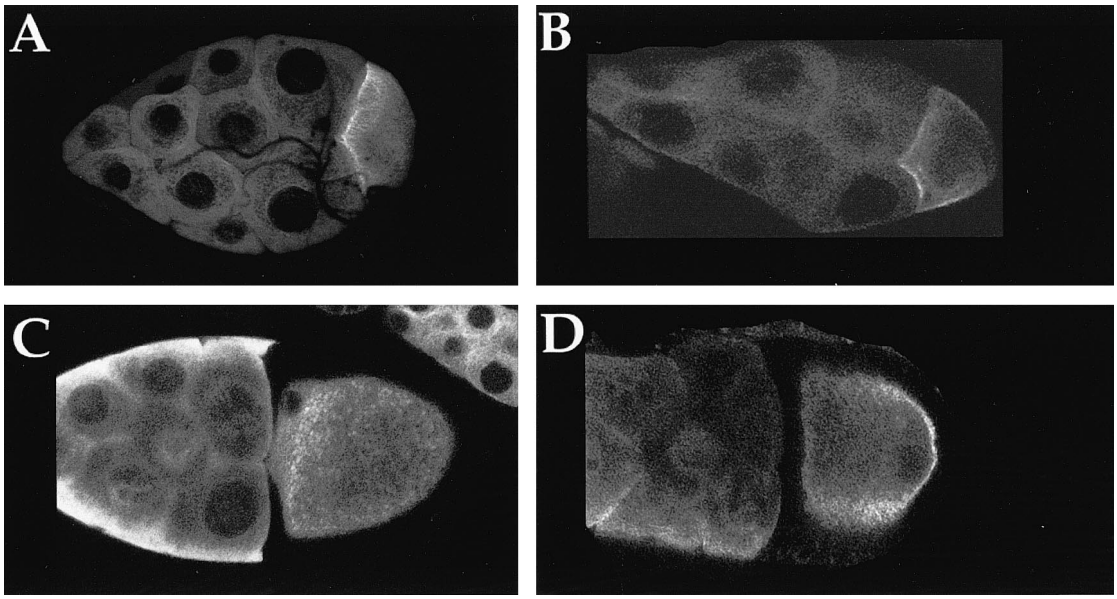

FIG. 5. *cnc* affects microtubule polarity. Confocal images showing the distribution of Tau-GFP expressed in the germline to visualise the microtubule network. (A, C) Wild-type stage 8 (A) and stage 10 (C). (B, D) cnc^{03921} mutant egg chambers. (B) Stage 8. The microtubule polarity is like in wild type. (D) In late stage 9, the microtubule density decreases anteriorly and increases posteriorly compared to wild type (C).

tures, like the dorsal appendages, nor for the DV embryonic axis.

DISCUSSION

cnc is a complex locus coding for three protein isoforms (CncA, CncB, CncC) which share a basic-leucine zipper domain at the carboxy terminus (McGinnis *et al.,* 1998). While CncA and CncC are expressed ubiquitously, CncB is expressed specifically in the head region of early embryos where it is required for the repression of *deformed* function and the formation of intercalary and labral structures (McGinnis *et al.,* 1998). Recently, double-stranded RNA interference experiments have shown that CncA and CncC are dispensible for embryonic development (Veraksa *et al.,* 2000). The two P-insertions used in this study affect all three isoforms. CncB is not expressed during oogenesis (McGinnis *et al.,* 1998), thus the mutant phenotypes we observed are due to a lack of either CncA, CncC, or both isoforms. Judging from their structure, both proteins probably function as transcription factors, as has been demonstrated for CncB and the Cnc homologs of vertebrates and other invertebrates. At present, no genes are known to be regulated by Cnc proteins during oogenesis. However, the *cnc* phenotype reveals two new aspects as to how DV polarity is established during oogenesis. (1) The initial asymmetric movement of the oocyte nucleus has to be followed by a separate process of stable anchoring of the nucleus at the anterior cortex. (2) An early pulse of asymmetric EGF signalling is insufficient to induce stable DV follicle cell patterning, indeed EGF receptor activation by Gurken has to persist until stage 10A to establish the DV axis of the *Drosophila* egg.

Nuclear Movement Is Followed by a Separate Process of Anterior Anchoring

In *cnc* mutant egg chambers, nuclear movement occurs normally. The nucleus remains cortically localised even after its posterior displacement. Since interference with components of the dynactin complex leads to the dissociation of the nucleus from the cortex (A.G. and S.R., unpublished results), we believe that the dynactin complex is not affected by the loss of *cnc* function.

However, the polarisation of the microtubule network is aberrant in stage 10A *cnc* oocytes. Higher numbers of microtubules accumulate in the posterior region of the oocyte at the expense of the anterior cortical ring which dominates the microtubule network of wild-type stage-9 to -10A egg chambers (Fig. 5). This second microtubule reorganisation could either be the cause of or result from the late displacement of the nucleus. In the first case, *cnc* would be required for a process that stabilises and maintains the microtubule polarity after stage 8. Prolonged signalling from posterior follicle cells might be necessary to suppress the reestablishment of MTOCs at the posterior pole. The reception of such a signal or its transmission to the cytoplasm might be impaired in the absence of *cnc* function. In this model, the reassembly of MTOCs in

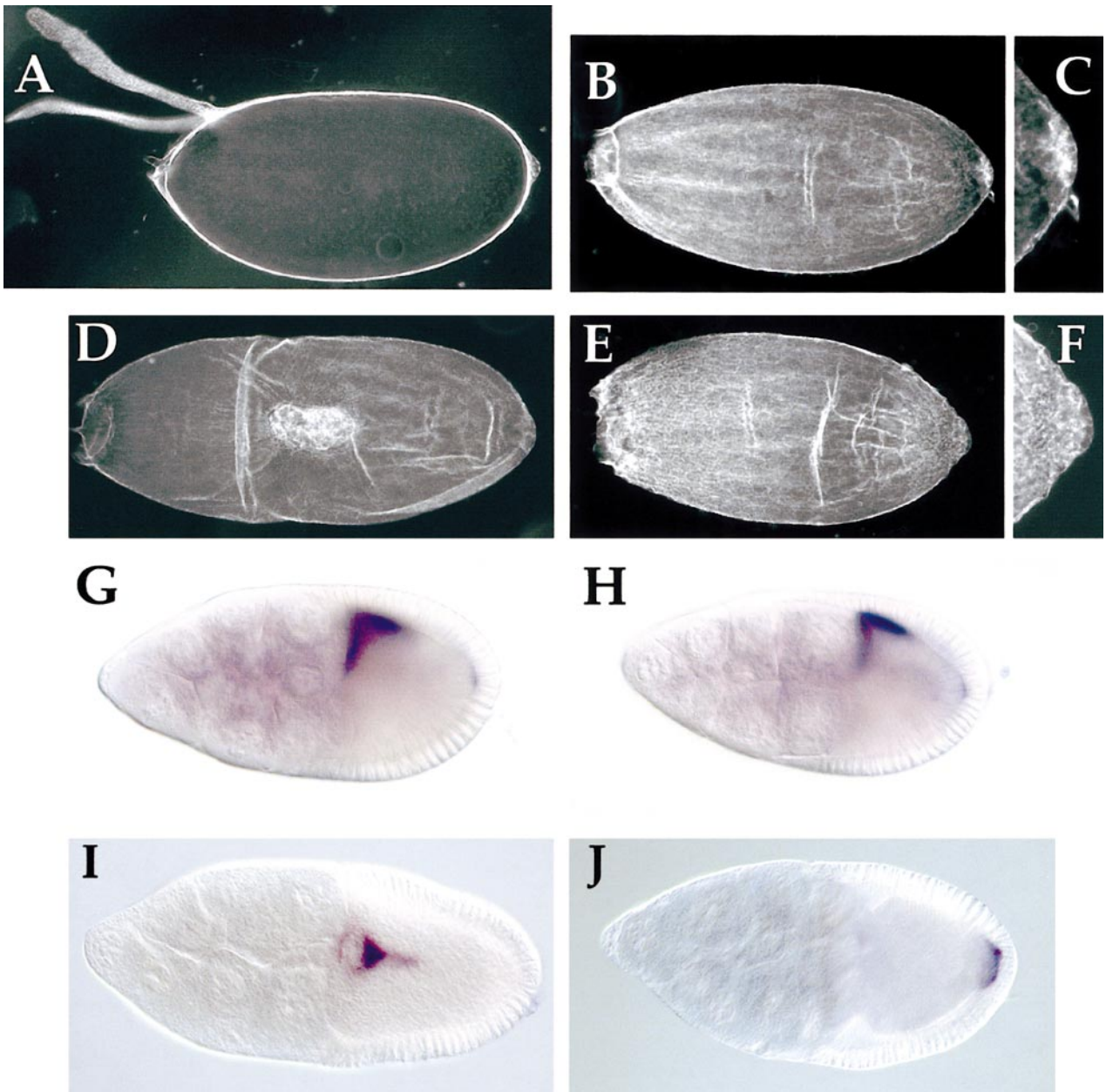

FIG. 6. *cnc* mutant eggs are ventralised. (A–F) Dark-field micrographs of eggs. (A) Wild-type egg. (B, D, E) Ventralised cn^{c03921} mutant eggs. (C, F) Magnified views of the aeropyle of the eggs shown in (B) and (E). The aeropyle in (F) is larger than in wild-type and similar to that found in *mago* mutant eggs (Micklem *et al.,* 1997). (G–J) Grk protein in stage-9 (G, H) and stage-10 (I, J) egg chambers. (G, I) Wild type. (H, J) cnc^{03921} mutant. At stage 9, Grk protein amount and localisation in *cnc* mutant egg chambers is indistinguishable from wild-type. At stage 10, Grk protein is associated with the posteriorly mislocalised nucleus in *cnc* mutant egg chambers.

posterior regions would lead to the redistribution of free tubulin and consequently weaken anterior MTOCs. The nucleus would subsequently migrate towards these ectopic posterior MTOCs. *bcd* mRNA also would become mislocalised since it is known to move, like the nucleus, towards the minus ends of microtubules, i.e., towards the MTOCs.

In the other scenario, *cnc* would be required specifically for oocyte nucleus anchoring at the anterior cortex. Anterior anchoring might be necessary since there is a massive influx of cytoplasm from the nurse cells to the anterior pole of the oocyte during egg chamber growth. If the nucleus is not properly anchored, these transport processes might

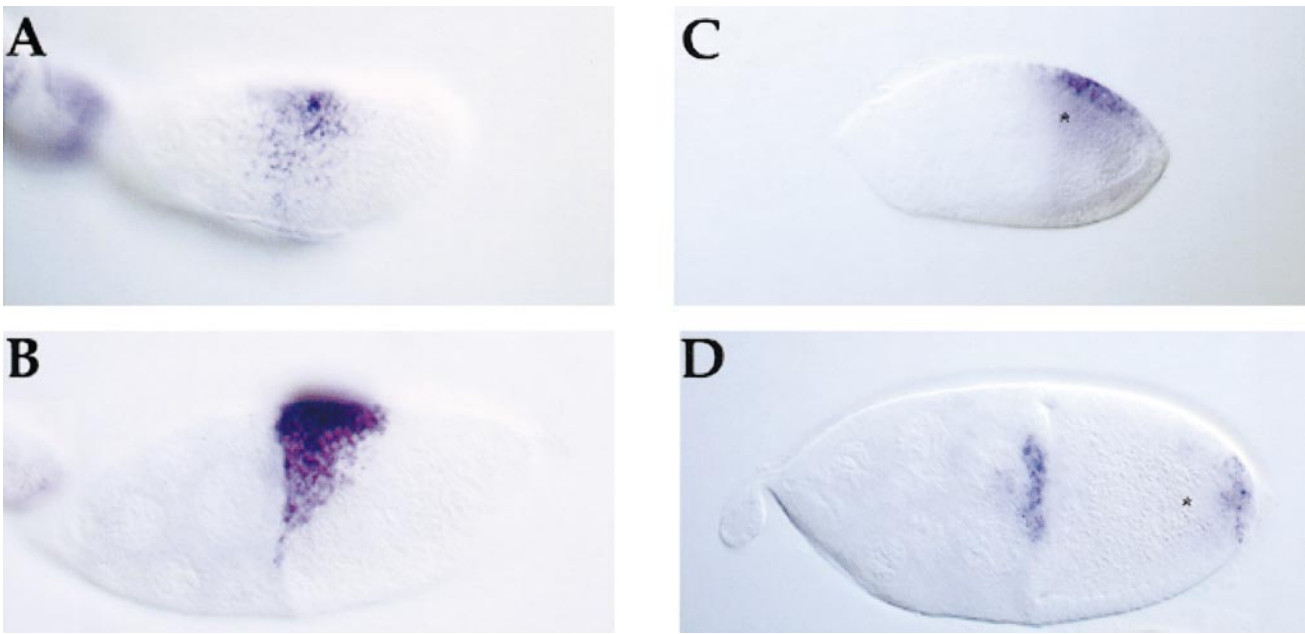

FIG. 7. *kekkon1* expression is initiated dorsally, but not maintained in *cnc* egg chambers. (A–D) *kek1* mRNA distribution. (A, B) Wild-type egg chambers. (C, D) cnc^{05561} mutant egg chambers. (A, C) Stage 9. (B, D) Stage 10A. (A–C) Lateral view. (D) Dorsal view. (C) *kek1* expression is initiated dorsaly in *cnc* mutant egg chambers at stage 9. The asterisk marks the oocyte nucleus which is correctly positioned at the dorsal–anterior corner of the oocyte. (D) Only a small patch of *kek1* expression remains dorsally in *cnc* mutant egg chambers at stage 10A. A new side of expression is sometimes seen in the vicinity of the mispositioned nucleus (asterisk).

dislodge the nucleus from the anterior pole. Why would this mispositioning of the nucleus lead to the reorganisation of the microtubule network? Such microtubule reorganisations have not been described in other mutant backgrounds where the nucleus does not reach the anterior cortex, such as *grk*, *cni*, *mago*, and *DLis-1* similar (González-Reyes *et al.,* 1995; Lei and Warrior, 2000; Micklem *et al.,* 1997; Newmark *et al.,* 1997; Roth *et al.,* 1995; Swan *et al.,* 1999). It has been shown that the nucleus gets encaged by microtubules when it arrives at the anterior pole in wild-type oocytes, indicating that the anteriorly localised nucleus acquires a microtubule-nucleating activity (Theurkauf *et al.,* 1992). This activity might remain associated with the mispositioned nucleus in *cnc* egg chambers and might subsequently cause the increased microtubule density in the posterior half of the *cnc* oocytes.

In both scenarios, the mislocalisation of *osk* remains somehow enigmatic. *osk* should not localise to the same region to which *bcd* is transported. However, normal *osk* transport from the anterior to the posterior might just be blocked by the mispostioned nucleus and its associated microtubules. Thus *osk* might be trapped in the vicinity of the ectopic nucleus on its way to the posterior pole.

Although at present it is impossible to distinguish between these two explanations for the *cnc* phenotype, the observed correlation between ectopic microtubules and nuclear position supports the second scenario. We therefore propose that two processes of oocyte nucleus anchoring can be distinguished: the general anchoring to the oocyte cortex and the spatially restricted anchoring to the anterior surface of the oocyte. The first process involves the dynein/dynactin complex, which controls cortical anchoring and nuclear movement. This process is likely to be unaffected in *cnc*. The second process is required after migration to tether the nucleus to the anterior surface of the oocyte. It keeps the nucleus in place during the growth of the oocyte after stage 9 of oogenesis. This process might be affected in *cnc* mutant egg chambers. As a transcription factor, Cnc might control the expression of proteins which function as components of the anterior anchor.

Signal Integration during Follicle Cell Patterning

In *cnc* egg chambers, the nucleus is first correctly localised to the anterior dorsal cortex of the oocyte and deviates from this position only at the end of stage 9. Although the nucleus remains attached to the cortex, its position is virtually randomised at stage 10 with respect to the AP axis of the egg. This phenotype is distinct from that caused by colchicine treatment or by pulses of heat shock-*cornichon* (Ghiglione *et al.,* 1999; Peri and Roth, 1999). In these situations, incomplete nuclear migration prevents the nucleus associated with *grk* mRNA from reaching the anterior pole. Therefore, the nucleus remains at the same

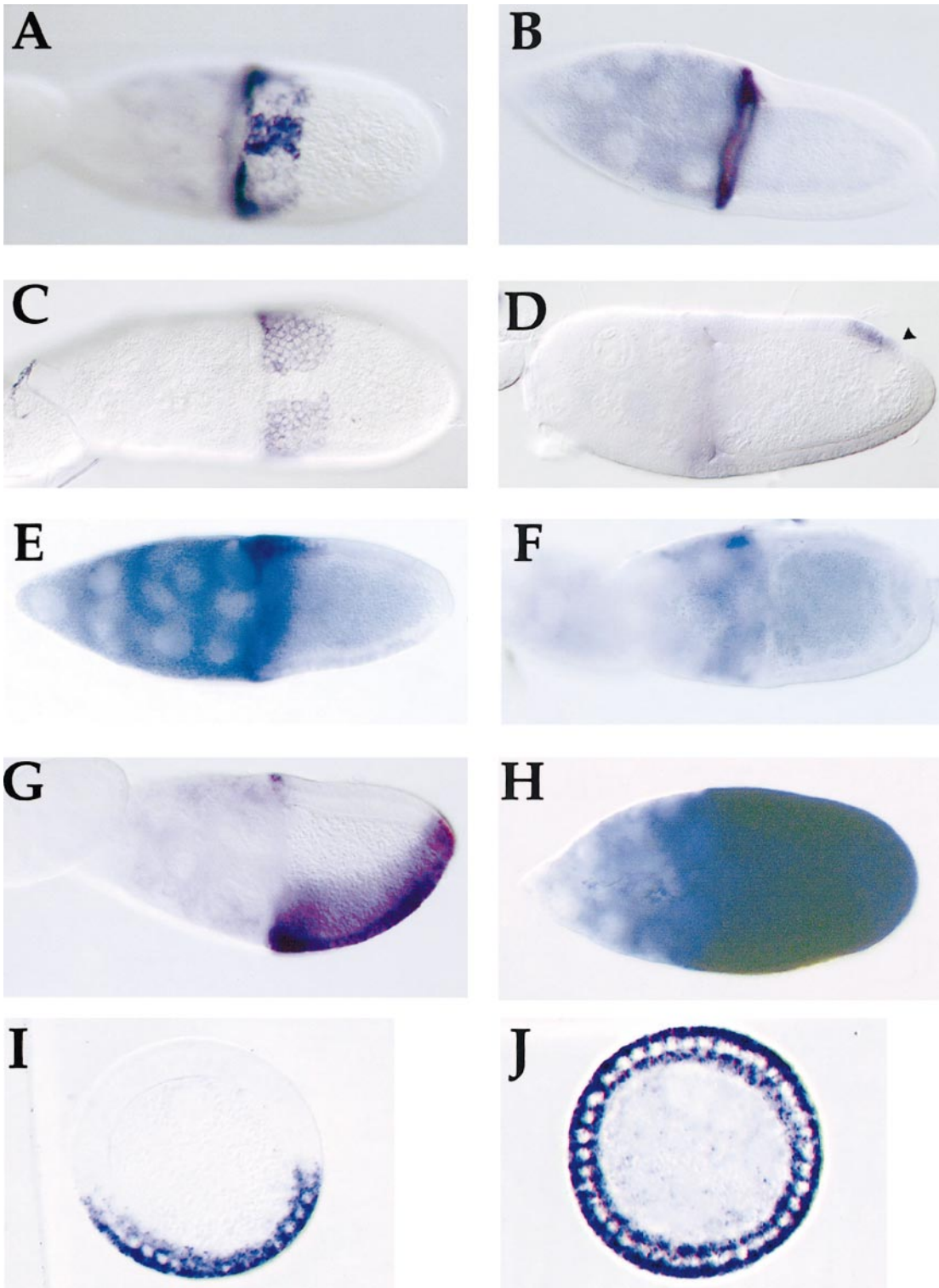

FIG. 8. Follicle cell paterning in *cnc* mutant egg chambers. (A, C, E, G, I) Wild-type stage-10A egg chambers. (B, D, F, H, J) cnc^{05561} mutant stage-10A egg chambers. (A, C) Dorsal views. (B, D, E–H) Lateral views. (I, J) Transverse sections at the postion of the oocyte nucleus. (A, B) *rho* mRNA distribution. *rho* is not expressed dorsally, but only in centripetal follicle cells of *cnc* mutant egg chambers. (C, D) *BR-C* mRNA distribution. *BR-C* is not expressed dorsally in *cnc* mutant egg chambers. In rare cases, *BR-C* is ectopically expressed in the vicinity of the mispositioned nucleus (arrowhead). (E, F) *mirror* mRNA distribution. *mirror* is not expressed in the follicle cells of *cnc* mutant egg chambers. (G–J) *pipe* mRNA distribution. *pipe* is uniformly expressed in *cnc* egg chambers.

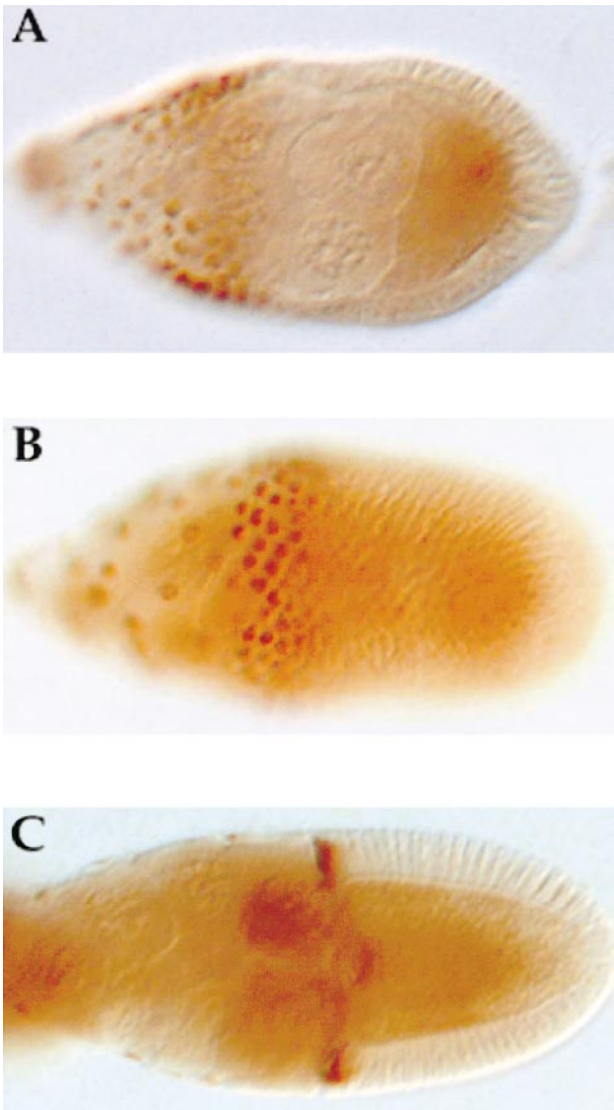

FIG. 9. Anterior-to-posterior migration of follicle cells transducing Dpp signalling. Dpp signalling in wild-type egg chambers was detected by using antibodies specific to the phosphorylated form of Mad. (A) Stage 8. High levels of Dpp signalling occur in a ring of follicle cells positioned anteriorly to the nurse cell/oocyte boundary. (B) Stage 9. The ring of follicle cells with high-level Dpp signalling moves posteriorly. (C) Stage 10A. The highest levels of Dpp signalling are found in centripetal follicle cells at the nurse cell/oocyte boundary.

ectopic position for extended periods and from there Grk signals to the overlying follicle cells. This might explain why strong ectopic *kek1* expression can be seen after colchicine treatment (or *hs-cni* pulses) while *kek1* expression was rarely observed at the side of the misplaced nucleus in *cnc* egg chambers. The *cnc* phenotype might be similar to that occasionally observed in *Bic-D*^{mom} mutant egg chambers where the nucleus seems to move in stage-10 oocytes (Swan and Suter, 1998). However, in this case, it is not clear whether the nucleus is correctly localised at stage 9 and whether it remains cortically localised during later stages.

Since the oocyte nucleus is localised correctly during stage 9 in *cnc* egg chambers, *cnc* provides an opportunity to test how the duration of spatially correct Grk signalling influences the DV patterning of the follicular epithelium. Our results suggest that asymmetric DER activation during stage 9 monitored by the expression of *kek1* is insufficient to establish a stable pattern of distinct follicle cell types along the DV axis. The lack of stable follicle cell patterning might have three reasons. First, signal duration and amplitude have been shown to influence the cellular response to activation by receptor tyrosin kinases. For example, in PC12 cells, short EGFR activation stimulates proliferation while sustained signalling promotes neuronal differentiation (Marshall, 1995). Likewise, in our case, follicle cell differentiation might require prolonged DER activation which cannot be supplied if the nucleus is not stably anchored at the anterior pole. Second, from stage 8 to 10A of oogenesis, follicle cells thin out over the nurse cells and migrate posteriorly to form a high columnar epithelium surrounding the oocyte. Thus, during this period, the identity of the follicle cells which pass the oocyte nucleus constantly changes. If the nucleus leaves its anterior position before stage 10A, the cells that migrate later are likely to receive less Grk signal than in wild type. This might lead to an anterior ventralisation of the egg, but can hardly account for the almost complete lack of follicle cell patterning exhibited by *cnc* egg chambers. Therefore, we favour the third explanation which stresses the importance of combinatorial control in follicle cell patterning. TGFβ and EGF signalling cooperate to establish dorsal follicle cell fates and their correct patterning (Dobens *et al.,* 2000; Peri and Roth, 2000). This cooperation might only be possible if the nucleus remains tightly connected to the anterior cortex. In *cnc* egg chambers, the Grk and Dpp sources may not come close enough to each other since the nucleus leaves its anterior position before the cells receiving high Dpp stimulation reach the nurse cell/oocyte boundary (Fig. 9). In this context, it is interesting to note that also the positive feedback of DER signalling initiated in the follicular epithelium has been shown to be under combinatorial control of Grk and Dpp (Peri and Roth, 2000). Activation of both signalling pathways in follicle cells induces *rho* which promotes the processing of a second TGFα-like ligand Spitz that can activate DER (Wasserman and Freeman, 1998). Such a mechanism could be started by a small pulse of Grk and later maintain itself. However, this does not occur in *cnc* egg chambers, suggesting that sufficient Dpp to initiate the postive feedback is only present at the nurse cell/oocyte boundary from late stage 9 onward. The observation that *mirror,* like *BR-C* and *rho*, is absent in *cnc* egg chambers suggests that the expression of *mirror* may also depend on both signals. Thus, the interaction of the dorsal Grk signal

with signals emerging from anterior follicle cells is likely to be crucial for all major aspects of DV follicle cell patterning.

ACKNOWLEDGMENTS

We thank Norbert Perrimon for providing a collection of lethal P element insertions, Jim Mohler for the gift of fly stocks, and Nadine McGinnis and Peter ten Dijke for providing antibodies. We thank Oliver Karst and Bianca Priester for help with fly work, and Robert Wilson, Sajith Dass, Jens Januschke, and Darren Gilmour for reading and discussing the manuscript. A.G. was supported by an EMBO fellowship. F.P. and O.K. were supported by grants from the DFG (SFB 243, 446, and 572).

REFERENCES

Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M., and Nusslein-Volhard, C. (1988). The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO J.* **7,** 1749–1756.

Bier, E., Jan, L. Y., and Jan, Y. N. (1990). Rhomboid a gene required for dorsoventral axis establishment and peripheral nervous system development in *Drosophila melanogaster*. *Genes Dev.* **4,** 190–203.

Chou, T. B., Noll, E., and Perrimon, N. (1993). Autosomal P(ovo-D1) dominant female-sterile insertions in *Drosophila* and their use in generating germ-line chimeras. *Development* **119,** 1359–1369.

Deng, W. M., and Bownes, M. (1997). Two signalling pathways specify localised expression of the *Broad-Complex* in *Drosophila* eggshell patterning and morphogenesis. *Development* **124,** 4639–4647.

Dobens, L. L., Peterson, J. S., Treisman, J., and Raftery, L. A. (2000). *Drosophila bunched* integrates opposing DPP and EGF signals to set the operculum boundary. *Development* **127,** 745–754.

Driever, W., and Nüsslein-Volhard, C. (1988). The Bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* **54,** 95–104.

Efimov, V., and Morris, N. (1998). A screen for dynein synthetic lethals in *Aspergillus nidulans* identifies spindle assembly checkpoint genes and other genes involved in mitosis. *Genetics* **8,** 1013–1018.

Ephrussi, A., Dickinson, L. K., and Lehmann, R. (1991). Oskar organizes the germ plasma and directs localization of the posterior determinant nanos. *Cell* **66,** 37–50.

Faulkner, N. E., Dujardin, D. L., Tai, C. Y., Vaughan, K. T., O'Connell, C. B., Wang, Y., and Vallee, R. B. (2000). A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function. *Nat. Cell Biol.* **2,** 784–791.

Geiser, J. R., Schott, E. J., Kingsbury, T. J., Cole, N. B., Totis, L. J., Bhattacharyya, G., He, L., and Hoyt, M. A. (1997). *Saccharomyces cerevisiae* genes required in the absence of the CIN8-encoded spindle motor act in functionally diverse mitotic pathways. *Mol. Biol. Cell* **8,** 1035–1050.

Ghiglione, C., Carraway, K. L., 3rd, Amundadottir, L. T., Boswell, R. E., Perrimon, N., and Duffy, J. B. (1999). The transmembrane molecule Kekkon 1 acts in a feedback loop to negatively regulate the activity of the *Drosophila* EGF receptor during oogenesis. *Cell* **96,** 847–856.

González-Reyes, A., Elliott, H., and St. Johnston, D. (1995). Polarization of both major body axes in Drosophila by *gurken-torpedo* signalling. *Nature* **375,** 654–658.

González-Reyes, A., and St. Johnston, D. (1994). Role of oocyte position in establishment of anterior–posterior polarity in *Drosophila*. *Science* **266,** 639–642.

González-Reyes, A., and St. Johnston, D. (1998). Patterning of the follicle cell epithelium along the anterior-posterior axis during *Drosophila* oogenesis. *Development* **125,** 2837–2846.

Holzbaur, E. L., and Vallee, R. B. (1994). Dyneins: Molecular structure and cellular function. *Annu. Rev. Cell Biol.* **10,** 339–372.

Jordan, K. C., Clegg, N. J., Blasi, J. A., Morimoto, A. M., Sen, J., Stein, D., McNeill, H., Deng, W. M., Tworoger, M., and Ruohola-Baker, H. (2000). The homeobox gene mirror links EGF signalling to embryonic dorso-ventral axis formation through Notch activation. *Nat. Genet.* **24,** 429–433.

Karki, S., and Holzbaur, E. L. (1999). Cytoplasmic dynein and dynactin in cell division and intracellular transport. *Curr. Opin. Cell Biol.* **11,** 45–53.

Kim-Ha, J., Smith, J. L., and Macdonald, P. M. (1991). *oskar* mRNA is localized to the posterior pole of the *Drosophila* oocyte. *Cell* **66,** 23–35.

Koch, E. A., and Spitzer, R. H. (1983). Multiple effects of colchicine on oogenesis in Drosophila: Induced sterility and switch of potential oocyte to nurse-cell developmental pathway. *Cell Tissue Res.* **228,** 21–32.

Lane, M. E., and Kalderon, D. (1994). RNA localisation along the anteroposterior axis of the *Drosophila* oocyte requires PKA-mediated signal to direct normal microtubule organization. *Genes Dev.* **8,** 2986–2995.

Lei, Y., and Warrior, R. (2000). The *Drosophila Lissencephaly1* (DLis1) gene is required for nuclear migration. *Dev. Biol.* **226,** 57–72.

Marshall, C. J. (1995). Specificity of receptor tyrosine kinase signaling: Transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80,** 179–185.

McGinnis, N., Ragnhildstveit, E., Veraksa, A., and McGinnis, W. (1998). A cap 'n' collar protein isoform contains a selective Hox repressor function. *Development* **125,** 4553–4564.

McNeill, H., Yang, C. H., Brodsky, M., Ungos, J., and Simon, M. A. (1997). mirror encodes a novel PBX-class homeoprotein that functions in the definition of the dorsal-ventral border in the Drosophila eye. *Genes Dev.* **11,** 1073–1082.

Micklem, D. R., Dasgupta, R., Elliott, H., Gergely, F., Davidson, C., Brand, A., Gonzalez-Reyes, A., and St. Johnston, D. (1997). The *mago nashi* gene is required for the polarisation of the oocyte and the formation of perpendicular axes in *Drosophila*. *Curr. Biol.* **7,** 468–478.

Mohler, J., Mahaffey, J. W., Deutsch, E., and Vani, K. (1995). Control of *Drosophila* head segment identity by the bZIP homeotic gene *cnc*. *Development* **121,** 237–247.

Neuman-Silberberg, F. S., and Schüpbach, T. (1993). The Drosophila dorsoventral patterning gene *gurken* produces a dorsally localized RNA and encodes a TGF-alpha-like protein. *Cell* **75,** 165–174.

Newmark, P. A., Mohr, S. E., Gong, L., and Boswell, R. E. (1997). Mago nashi mediates the posterior follicle cell-to-oocyte signal to organize axis formation in Drosophila. *Development* **124,** 3197–3207.

Peri, F., Bökel, C., and Roth, S. (1999). Local Gurken signaling and dynamic MAPK activation during *Drosophila* oogenesis. *Mech. Dev.* **81,** 75–88.

Peri, F., and Roth, S. (2000). Combined activities of Gurken and Decapentaplegic specify dorsal chorion structures of the *Drosophila* egg. *Development* **127,** 841–850.

Perrimon, N., Lanjuin, A., Arnold, C., and Noll, E. (1996). Zygotic lethal mutations with maternal effect phenotypes in *Drosophila melanogaster*. II. Loci on the second and third chromosomes identified by P-element-induced mutations. *Genetics* **144,** 1681–1692.

Persson, U., Izumi, H., Souchelnytskyi, S., Itoh, S., Grimsby, S., Engstrom, U., Heldin, C. H., Funa, K., and ten Dijke, P. (1998). The L45 loop in type I receptors for TGF-beta family members is a critical determinant in specifying Smad isoform activation. *FEBS Lett.* **434,** 83–87.

Queenan, A. M., Barcelo, G., Van Buskirk, C., and Schüpbach, T. (1999). The transmembrane region of Gurken is not required for biological activity, but is necessary for transport to the oocyte membrane in *Drosophila*. *Mech. Dev.* **89,** 35–42.

Raftery, L. A., Twombly, V., Wharton, K., and Gelbart, W. M. (1995). Genetics screens to identify elements of the *decapentaplegic* signaling pathway in *Drosophila*. *Genetics* **139**, 241–254.

Roth, S., Jordan, P., and Karess, R. (1999). Binuclear *Drosophila* oocytes: consequences and implications for dorsal-ventral patterning in oogenesis and embryogenesis. *Development* **126,** 927–934.

Roth, S., Neuman-Silberberg, F. S., Barcelo, G., and Schüpbach, T. (1995). Cornichon and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in Drosophila. *Cell* **81,** 967–978.

Saunders, C., and Cohen, R. S. (1999). The role of oocyte transcription, the 5′UTR, and translation repression and derepression in *Drosophila gurken* mRNA and protein localization. *Mol. Cell* **3,** 43–54.

Schüpbach, T. (1987). Germ line and soma cooperate during oogenesis to establish the dorsoventral pattern of egg shell and embryo in *Drosophila melanogaster*. *Cell* **49,** 699–708.

Sen, J., Goltz, J. S., Stevens, L., and Stein, D. (1998). Spatially restricted expression of pipe in the *Drosophila* egg chamber defines embryonic dorsal-ventral polarity. *Cell* **95,** 471–481.

Smith, D. S., Niethammer, M., Ayala, R., Zhou, Y., Gambello, M. J., Wynshaw-Boris, A., and Tsai, L. H. (2000). Regulation of cytoplasmic dynein behaviour and microtubule organization by mammalian Lis1. *Nat. Cell Biol.* **2,** 767–775.

St. Johnston, D., and Nüsslein-Volhard, C. (1992). The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68,** 201–219.

Swan, A., and Suter, B. (1996). Role of *Bicaudal-D* in patterning the *Drosophila* egg chamber in mid-oogenesis. *Development* **122,** 3577–3586.

Swan, A., Nguyen, T., and Suter, B. (1999). *Drosophila Lissencephaly*-1 functions with Bic-D and dynein in oocyte determination and nuclear positioning. *Nat. Cell Biol.* **1,** 444–449.

Tanimoto, H., Itoh, S., ten Dijke, P., and Tabata, T. (2000). Hedgehog creates a gradient of DPP activity in Drosophila wing imaginal discs. *Mol. Cell* **5,** 59–71.

Tautz, D., and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals translational control of the segmentation gene hunchback. *Chromosoma* **98,** 81–85.

Theurkauf, W. E. (1994). Microtubules and cytoplasm organization during Drosophila oogenesis. *Dev. Biol.* **165,** 352–360.

Theurkauf, W. E., Smiley, S., Wong, M. L., and Alberts, B. M. (1992). Reorganization of the cytoskeleton during *Drosophila* oogenesis implications for axis specification and intercellular transport. *Development* **115,** 923–936.

Tomancak, P., Guichet, A., Zavorszky, P., and Ephrussi, A. (1998). Oocyte polarity depends on regulation of gurken by Vasa. *Development* **125,** 1723–1732.

Twombly, V., Blackman, R. K., Jin, H., Graff, J. M., Padgett, R. W., and Gelbart, W. M. (1996). The TGF-beta signaling pathway is essential for *Drosophila* oogenesis. *Development* **122,** 1555–1565.

van Eeden, F., and St. Johnston, D. (1999). The polarisation of the anterior–posterior and dorsal–ventral axes during *Drosophila* oogenesis. *Curr. Opin. Genet. Dev.* **9,** 396–404.

Veraksa, A., McGinnis, N., Li, X., Mohler, J., and McGinnis, W. (2000). Cap 'n' collar B. cooperates with a small maf subunit to specify pharyngeal development and suppress deformed homeotic function in the drosophila head. *Development* **127,** 4023–4037.

Wasserman, J. D., and Freeman, M. (1998). An autoregulatory cascade of EGF receptor signaling patterns the Drosophila egg. *Cell* **95,** 355–364.

Wiersdorff, V., Lecuit, T., Cohen, S. M., and Mlodzik, M. (1996). Mad acts downstream of Dpp receptors, revealing a differential requirement for dpp signaling in initiation and propagation of morphogenesis in the Drosophila eye. *Development* **122,** 2153–2162.

Xiang, X., Osmani, A. H., Osmani, S. A., Xin, M., and Morris, N. R. (1995). *NudF*, a nuclear migration gene in *Aspergillus nidulans*, is similar to the human *LIS-1* gene required for neuronal migration. *Mol. Biol. Cell* **6,** 297–310.

Received for publication February 1, 2001
Revised April 25, 2001
Accepted June 5, 2001
Published online July 30, 2001